

WHAT IS CLAIMED IS:

- ✓ 1. A method comprising:
- 5 a) obtaining a DNA comprising an anchorable moiety;
- b) cleaving said DNA with a first restriction endonuclease;
- c) ligating a linker molecule to said DNA;
- d) immobilizing the DNA fragment that includes said anchorable moiety to
an anchor;
- 10 e) cleaving said immobilized DNA from said anchor with a second
restriction endonuclease;
- f) ligating a second linker molecule to said cleaved DNA;
- g) amplifying said ligated DNA.
2. The method of claim 1, wherein said DNA is immobilized prior to cleaving with
said first endonuclease.
- 15 3. The method of claim 1, wherein said DNA is non-genomic DNA.
4. The method of claim 1, wherein said DNA is cDNA.
- 20 5. The method of claim 1, wherein said anchorable moiety comprises a means of
adhering.
6. The method of claim 5, wherein said means of adhering comprises a means of
establishing a non-covalent interaction.
- 25 7. The method of claim 5, wherein said means of adhering comprises a means of
establishing a covalent interaction.
- 30 8. The method of claim 5, wherein said means of adhering comprises a ligand.

9. The method of claim 5, wherein said means of adhering is biotin.
10. The method of claim 5, wherein said means of adhering comprises an antibody.
- 5 11. The method of claim 1, wherein said anchorable moiety is located at the 3' end.
12. The method of claim 4, wherein said cDNA is reverse transcribed from messenger RNA.
- 10 13. The method of claim 12, wherein said reverse transcription is initiated at an oligo dT.
14. The method of claim 12, wherein said reverse transcription is initiated at a random hexamer.
- 15 15. The method of claim 13, wherein said oligo dT is biotinylated.
16. The method of claim 13 wherein said cDNA is immobilized on a substrate by means of said biotinylated oligo dT.
- 20 17. The method of claim 16, wherein said substrate is streptavidin.
18. The method of claim 1, wherein the order of said first and said second restriction endonucleases is reversed.
- 25 19. The method of claim 1, wherein said amplification is initiated at primers comprising a sequence complementary to said first and said second linkers respectively.
20. The method of claim 1, wherein said ligated DNA is amplified with a primer set comprising:

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- a) a first amplification primer, wherein the 5' sequence of said primer is complementary to said first linker sequence and the 3' sequence comprises a specificity region;
- b) a second amplification primer, wherein the 5' sequence of said primer is complementary to said second linker sequence and the 3' sequence comprises a specificity region.

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21. The method of claim 20, wherein said amplification is performed with an array of combinations of alternate amplification primers.

22. The method of claim 20 wherein said DNA fragment is preamplified.

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SUB A³ 23. The method of claim 1, further comprising, identifying the amplified DNA.

24. The method of claim 23, wherein said identification is based upon length.

25. The method of claim 23, wherein said identification is performed by a computer program.

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26. The method of claim 21, wherein said array of amplifications is performed in a multi-well plate.

27. The method of claim 20, wherein the specificity region of the primers of the first primer set is 3,4,5,6,7 or 8 base pairs long.

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28. The method of claim 20, wherein the specificity region of the primers of the second primer set is 3,4,5,6,7 or 8 base pairs long.

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SUB A⁴ 29. The method of claim 1, wherein said amplification comprises polymerase chain reaction, nucleic acid sequence based amplification, transcription mediated amplification, strand displacement amplification or ligase chain reaction.

30. The method of claim 1, wherein said first restriction endonuclease has a four base pair recognition site.

5 31. The method of claim 1, wherein said first restriction endonuclease has a recognition site of five, six, seven or eight base pairs.

32. The method of claim 30, wherein said first restriction endonuclease is NlaIII, DpnII, Sau3AI, Hsp92II, MboI, NdeII, Bsp1431, Tsp509 I, HhaI, HinP1I, HpaII, MspI, TaqalphaI, MaeII or K2091.

33. The method of claim 1, wherein said second restriction endonuclease has a four base pair recognition site.

15 34. The method of claim 1, wherein said second restriction endonuclease has a recognition site of five, six, seven or eight base pairs.

35. The method of claim 33, wherein the restriction endonuclease is NlaIII, DpnII, Sau3AI, Hsp92II, MboI, NdeII, Bsp1431, Tsp509 I, HhaI, HinP1I, HpaII, MspI, TaqalphaI, MaeII or K2091.

20 36. The method of claim 1, wherein a label is incorporated into said amplified DNA.

37. The method of claim 36, wherein said label is incorporated by means of a labeled primer.

25 38. The method of claim 36, further comprising, partial nucleotide sequence identification of the amplified products by the identity of the label.

30 39. The method of claim 36, wherein said label is a chromophore.

40. The method of claim 36, wherein said label is a fluorophore.
41. The method of claim 36, wherein said label is an affinity label.
- 5 42. The method of claim 36, wherein said label is a dye.
43. The method of claim 37, wherein the 5' end of said primer comprises an amino moiety and a fluorophore is covalently attached by the reaction of a succinimido ester of the fluorophore to the 5' amino-modified primer.
- 10 44. The method of claim 40, wherein said fluorophore is Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy2, Cy3, Cy5,6-FAM, Fluorescein, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, ROX, TAMRA, TET, Tetramethylrhodamine, and Texas Red.
- 15 45. The method of claim 1, wherein the products of said amplification are analyzed.
- 20 46. The method of claim 45, wherein said analysis of amplification products is by polyacrylamide gel electrophoresis.
47. The method of claim 45, wherein said analysis of amplification products is by capillary gel electrophoresis.
- 25 48. The method of claim 45, wherein said analysis of amplification products is by mass spectrophotometry.
- 30 49. The method of claim 45, wherein said analysis of amplification products is by energy transfer.

50. The method of claim 45, wherein said analysis of amplification products is by the BioStar technology.

5 51. The method of claim 45, wherein said analysis of amplification products is by the Luminex technology.

52. The method of claim 45, wherein said analysis of amplification products comprises quantifying amplification products.

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10 53. The method of claim 52, wherein said quantifying is by measuring the ratio of each amplified product to a co-amplified reference-gene.

54. The method of claim 52, wherein said quantifying is by measuring the ratio of each amplified product to a panel of co-amplified reference-genes.

15 55. The method of claim 52, wherein said analysis of amplification products is by Real-Time PCR.

20 56. The method of claim 45, wherein said analysis of amplification products is performed in a multi-well plate.

57. The method of claim 45, wherein said analysis of amplification products is performed on a membrane.

25 58. The method of claim 45, wherein said analysis of amplification products is performed on a solid matrice.

59. The method of claim 58, wherein said solid matrice is a DNA chip.

30 60. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a different cell or tissue.

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61. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cancerous cell or tissue.
- 5 62. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a pharmaceutical compound.
- 10 63. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a teratogenic compound.
64. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a carcinogenic compound.
- 15 65. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a toxic compound.
- 20 66. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a biological response modifier.
- 25 67. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a hormone, a hormone agonist or a hormone antagonist.
- 30 68. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a cytokine.
69. The method of claim 1, performed on DNA derived from a normal cell or tissue and on DNA derived from a cell or tissue treated with a growth factor.

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70. The method of claim 1, performed on DNA derived from a normal cell or tissue and on the DNA derived from a cell or tissue treated with the ligand of a known biological receptor.
- 5 71. The method of claim 1, performed on DNA derived from a cell or tissue type obtained from a different species.
72. The method of claim 1, performed on DNA derived from a cell or tissue type obtained from a different organism.
- 10 73. The method of claim 1, performed on DNA derived from a cell or tissue at different stages of development.
74. The method of claim 1, performed on DNA derived from a normal cell or tissue and on the DNA derived from a cell or tissue that is diseased.
- 15 75. The method of claim 1, performed on DNA derived from a cell or tissue cultured in vitro under different conditions.
- 20 76. The method of claim 1, performed on the DNA derived from a cell or tissue from two organisms of the same species with a known genetic difference.
- ✓ 77. A kit for detection of gene expression comprising:
- 25 a) a first restriction enzyme;
- b) a second restriction enzyme;
- c) a first, ligatable, oligonucleotide tag;
- d) a second, ligatable, oligonucleotide tag;
- e) a first amplification primer, wherein the 5' sequence of said primer is complementary to said first linker sequence and the 3' sequence comprises a specificity region;
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- f) a second amplification primer, wherein the 5' sequence of said primer is complementary to said second linker sequence and the 3' sequence comprises a specificity region;
- g) software capable of analyzing data generated from said kit.

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78. The kit of claim 77, wherein said first restriction endonuclease is a four base pair cutter.

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79. The kit of claim 78, wherein said first restriction endonuclease is NlaIII, DpnII, Sau3AI, Hsp92II, MboI, NdeII, Bsp143I, Tsp509 I, HhaI, HinP1I, HpaII, MspI, TaqalphaI, MaeII or K2091.

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80. The kit of claim 77, wherein said second restriction endonuclease is a four base pair cutter.

81. The kit of claim 80, wherein said second restriction endonuclease is NlaIII, DpnII, Sau3AI, Hsp92II, MboI, NdeII, Bsp143I, Tsp509 I, HhaI, HinP1I, HpaII, MspI, TaqalphaI, MaeII or K2091.

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82. The kit of claim 77, wherein said first primer set comprises the sequence GCTGTCTAGACG (SEQ ID NO:1).

83. The kit of claim 77, wherein said second primer set comprises the sequence CGGTGATGCATC (SEQ ID NO:2).

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84. The method of claim 1, wherein said anchorable moiety is located at the 5' end.

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